Original Article

日歯保存誌 63（1）：1〜13，2020

Study on Rehardening of Demineralized Dentin with the New Pulp-capping Agents Containing Bioactive Glass

SAWAI Kenshiro, YASUO Kenzo, KOMASA Reiko,
YOSHIKAWA Kazushi and YAMAMOTO Kazuyo

Department of Operative Dentistry, Osaka Dental University

Abstract

Purpose: We measured the Knoop hardness of demineralized dentin over time using Cariotester to determine the effectiveness of several types of pulp-capping agent for atraumatic indirect pulp capping（AIPC）.

Methods: Extracted human molars were used to prepare dentin samples with a diameter of 10 mm and thickness of 2 mm. Sound dentin samples were immersed in lactic acid solution and were regarded as demineralized when the value obtained using Cariotester was approximately 20 KNH. A new bioactive glass-compounded pulp-capping agent（Shofu）, Bio MTA cement（J. Morita）, and NEX MTA cement（GC）were used as the pulp-capping agents in the present study. Each pulp-capping agent was applied to the surface of demineralized dentin and covered with Base cement. After the Base cement solidified, pulp-capped dentin samples and controls were divided into two groups: those placed in a container with 100% humidity and those immersed in remineralization solution, and stored at 37℃ in a thermostatic chamber for l month and 3 months. The hardness of the capping agent-applied region was then measured. Data were analyzed using a one-way analysis of variance and Tukey's test（$\alpha=0.001$）. Pulp-capping agent-applied surfaces were also observed under SEM.

Results: The hardness of demineralized dentin increased with the application of a new bioactive glass-compounded pulp-capping agent, Bio MTA cement, and NEX MTA cement, and mineralized substance-like aggregates were deposited on the surface of and between collagen fibers. It is assumed that the demineralized dentin had remineralized as the bioactive glass-compounded pulp-capping agent was pasted.

Conclusion: These results indicate the effectiveness of the new bioactive glass-compounded pulp-capping agent in the remineralization of demineralized dentin.

Key words: Knoop hardness, pulp-capping agent, remineralization

Corresponding author: Dr. SAWAI, Department of Operative Dentistry, Osaka Dental University, 1-5-17, Otemae, Chuo-ku, Osaka 540-0008, Japan

TEL: +81-6-6910-1111, FAX: +81-6-6943-8051, E-mail: sawai-k@cc.osaka-dent.ac.jp

Received for Publication: November 15, 2019/Accepted for Publication: December 5, 2019

DOI: 10.11471/shikahozon.63.1

Introduction

In recent years, the concept of minimal intervention (MI) to save as much sound dentin as possible and minimize invasion has become widespread in the treatment of dental caries. Reasons for this include advances in pathological research on carious dentin, and the development of dentin-bonding resins. Fusayama et al.[1–3] reported that carious dentin could be classified into the outer layer in which the dentin has been demineralized and the collagen fiber disintegrated by cariogenic bacteria, and the inner layer in which cariogenic bacteria do not exist, even though the dentin is partially demineralized due to the effects of cariogenic bacteria. A liquid for detecting dental caries was developed as an indicator to distinguish between these two layers, and has been widely used for caries removal[4,5]. While the outer layer which becomes dyed by the liquid needs to be removed, it has been reported that physiological remineralization occurs in the inner layer, which is not dyed by the liquid, after repair to actively preserve it[6–8].

However, there are many cases in which pulp extirpation becomes unavoidable when caries advances to the deeper parts of the dentin adjacent to the dental pulp and when the total removal of infected dentin would result in pulp exposure[9]. In such cases, atraumatic indirect pulp capping (AIPC) is recommended, in which pulp extraction is avoided but instead preservation of the pulp is attempted by intentionally leaving the infected dentin adjacent to the pulp to paste a calcium hydroxide formulation or polycarboxylate cement combined with tannin-fluoride preparation, and promoting the sterilization and remineralization of the infected dentin that was left as well as the formation of tertiary dentin (reparative dentin). The remineralization of demineralized dentin by pasting a calcium hydroxide formulation or polycarboxylate cement combined with tannin-fluoride preparation has been examined by various methods including hardness examination, X-ray examination, bacteriological examination, and histopathological examination, and has been reported to be effective[10–17]. Although hardness is an indicator of special clinical importance, there had been few examinations using objective indicators until Matsuda et al.[18] reported on the results of measuring the hardness of carious dentin in the oral cavity over time after using a calcium hydroxide formulation or a pulp-capping agent combined with HY agent with Cariotester, an instrument for measuring the hardness of carious dentin developed by Shimizu et al.[19,20]. Their results showed that hardness that could be considered sound did not recover in 3 months, even though the demineralized dentin hardened depending on the Ca concentration. In this study, we therefore measured the hardness of demineralized dentin over time with Cariotester after using the new pulp-capping agent or MTA cement. We also pasted an improved pulp-capping agent on demineralized dentin, which we prepared by using lactic acid solution, to measure the Knoop hardness values of the pasted surfaces after 1 month and 3 months. This article also reports our examination on the effectiveness of the pulp-capping agent by observing SEM images of the surfaces pasted with the pulp-capping agent.

Materials and methods

1. Experiment samples

As the test teeth, we used human molar teeth that had been removed and stored frozen at −40℃ at the Oral Surgery Department, Osaka Dental University Hospital. We thawed the teeth under running water immediately before use, and excluded those with caries, clouding, coloring or cracking after observing the occlusal surface of each tooth with the naked eye.

The study was conducted after obtaining approval from the ethics committee, Graduate School of Dentistry, Osaka Dental University (Approval No. 111023, April 5, 2019).

2. Experiment methods

1) Hardness measurement

In order to examine the changes in hardness over time after pasting the pulp-capping agent, we used Cariotester (SUK-971, Saneime Co., Ltd., Kanagawa, Japan; hereafter "Cariotester") to measure the hardness of sound dentin, demineralized dentin, and dentin at 1 month or 3 months after pulp capping according to the manufacturer's instructions. We specified the measurement range to within 3 mm diameter of the center on the enamel side in the sound dentin samples, and within 3 mm diameter from the center of the decalcified part in demineralized samples and pulp-capping samples. We took measurements at five points in each sample, and

Table 1 Materials used

Brand	Code	Component	Manufacturer	Lot No.
The New Pulp-capping Agents containning Bioactive glass	SH-C	S-PRG filler, Purified water, etc.	Shofu	G3P-70
Bio MTA Cement	Bio-C	Powder：Calcium carbonate, Silicon dioxide, Aluminum oxide, Zirconia Liquid：Purified water	J. Morita	BM1711D11
NEX MTA Cement	NEX-C	Powder：Calcium oxide, Bismuth oxide, Silicon dioxide, Aluminum oxide Liquid：Purified water	GC	1808031
Base Cement	BC	Powder：Fluoroalumino silicate glass Liquid：Acrylic acid-Tricarboxylic acid copolymer solution, Tartaric acid	Shofu	Powder：011621 Liquid：031620

used the average value of the five points as the hardness of the sample. We specified the number of samples under each condition as three.

2）Preparation of samples

We removed the pulp by cutting the root of a human molar tooth at 3 mm to the apex from the anatomical cervical line, and cut the enamel crown part and root dentin part perpendicular to the tooth axis with a model trimmer. We prepared dentin samples with 10 mm diameter and 2 mm thickness by polishing the enamel side and the pulp cavity side of the exposed dentin with waterproof abrasive paper #1000（Trimite papers, Wingo Co., Ltd., Osaka, Japan）. We measured the hardness of the enamel side of the dentin samples with Cariotester, and used those that had an enamel side hardness of around 60 KHN as the sound dentin samples（hereafter,"sound samples"）.

3）Preparation of demineralized dentin

To decalcify the sound samples, we used lactic acid (Kishida Chemical Co., Ltd., Osaka, Japan; hereafter "lactic acid"), which is one of the major organic acids that are produced by cariogenic bacteria.

We soaked the enamel sides of the sound samples in 50 m*l* of 20 mol/*l* lactic acid solution, and left them standing for 10 hours under suction from the pulp cavity side at 0.01 MPa using an Aspirator（MDA-006, Ulvac, Inc., Kanagawa, Japan; hereafter "Aspirator"）. After thoroughly rinsing the sound samples with distilled water, we measured the hardness of the enamel side with Cariotester, and used those with a hardness value of around 20 KHN as the demineralized dentin samples.

4）Preparation of pulp-capping samples

Table 1 lists the pulp-capping agents and cements that we used in the experiments. We tested Bio MTA cement (J. Morita, Osaka, Japan; hereafter "Bio-C") and NEX MTA cement (GC, Tokyo, Japan; hereafter "NEX-C") according to the manufacturer's instructions as commercially available MTA cement products. We also used a new bioactive glass-compounded pulp-capping agent (Shofu Inc., Kyoto, Japan; hereafter "SH-C") which has a different mechanism from MTA cements. We pasted thinly each of these pulp-capping agents to the surface of the decalcified part of the demineralized dentin sample, and covered it with Base cement (Shofu Inc., Kyoto, Japan; hereafter "BC") after the pulp-capping agent had hardened, then used the samples as the pulp-capping samples. We also used demineralized dentin samples which were covered only with BC instead of pasting the pulp-capping agent as a control. After BC curing, we placed the pulp-capping samples and control samples in a container with 100% humidity, designating these as the distilled water group, and immersed the two types of samples in remineralization solution（1.5 mmol/*l* $CaCl_2$, 0.9 mmol/*l* KH_2PO_4, 130 mmol/*l* KCl, and 20 mmol/*l* HEPES）adjusted to pH7.0 with KOH, designating these as the remineralization solution group, and stored them in a 37℃ thermostatic chamber for 1 month or 3 months. After the storage period, we removed the BC and the pulp-capping agent from the pulp-capping samples and control samples while being careful not to touch the area with a cutting instrument such as a probe where the pulp-capping agent was pasted, and measured the hardness of the area where

Table 2 Knoop hardness of dentin in each condition after 1 month and 3 months

Stored condition	Code	SH-C	Bio-C	NEX-C	BC
Distilled water	Sound dentin	64.0 (0)	63.2 (1.4)	64.0 (0)[a]	63.6 (0.7)
	Demineralized dentin	21.6 (0.7)	22.0 (1.1)	20.7 (0.2)	20.6 (1.2)[b]
	1M	45.1 (1.8)	47.3 (0.7)	56.1 (1.6)[a]	25.2 (2.5)[b]
	Sound dentin	64.0 (0)[c]	63.2 (1.4)[d]	64.0 (0)[e]	63.6 (0.7)
	Demineralized dentin	19.6 (0.4)	20.1 (0.6)	19.8 (0.4)	21.1 (1.0)[f]
	3M	59.0 (7.1)[c]	61.2 (3.1)[d]	64.0 (0)[e]	22.4 (0.4)[f]
Remineralization solution	Sound dentin	64.0 (0)	63.7 (0.5)	61.6 (2.4)[g]	62.0 (1.8)
	Demineralized dentin	19.8 (0.1)	20.6 (1.5)	21.3 (0.2)	22.1 (0.7)[h]
	1M	46.8 (2.5)	50.3 (3.7)	53.1 (6.1)[g]	25.1 (1.7)[h]
	Sound dentin	64.0 (0)[i]	63.7 (0.5)[j]	61.6 (2.4)[k]	62.0 (1.8)
	Demineralized dentin	20.1 (0.6)	20.4 (0.7)	20.1 (0.4)	21.2 (0.6)[l]
	3M	60.1 (2.0)[i]	62.8 (1.7)[j]	64.0 (0)[k]	22.3 (0.1)[l]

The unit Knoop hardness of dentin in each condition.( )means SD of Knoop hardness. The number of samples was three for each condition. In each group, values with the same superscript letters are no significantly different ($\alpha = 0.001$).

the agent had been pasted.

3. Observation of SEM images

To observe the surface of the pulp-capping sample after hardness measurement, we fixed and dehydrated the sample with alcohol series according to the normal method, and freeze-dried the sample using a t-butyl alcohol freeze dryer (VFD21S, VD, Ibaraki, Japan). Then we conducted Os vapor deposition using an osmium coater (HPC-20, VD, Ibaraki, Japan), and observed the SEM images using a field emission scanning electron microscope (S-4800, Hitachi, Ltd., Tokyo, Japan; hereafter "SEM"). We also observed the enamel side surfaces of sound samples and demineralized samples in a similar fashion.

4. Statistical processing

We statistically analyzed the measurement values obtained from the samples by one-way analysis of variance and Tukey's test ($\alpha < 0.001$).

Results

1. Hardness measurement

Table 2 shows the hardness value for each sample we measured using Cariotester.

1) Hardness of sound samples and demineralized samples

The average hardness of sound samples was 63.3±1.2 KHN, and the average hardness of demineralized samples was 20.7±1.2 KHN. For all samples, hardness decreased significantly in the demineralized sample compared to the sound one.

2) Hardness of pulp-capping samples

(1) SH-C pasting group

This section outlines the results for the SH-C pasting group. The hardness value after 1 month and 3 months was 45.1±1.8 KHN and 59.0±7.1 KHN in the distilled water group, and was 46.8±2.5 KHN and 60.1±2.0 KHN in the remineralization solution group, respectively.

In the SH-C pasting group, hardness improved significantly after 1 month and after 3 months compared to the demineralized sample, in both the distilled water group and the remineralization solution group. Furthermore, it improved to a hardness level where there was no significant difference from the sound sample after 3 months in both groups.

(2) Bio-C pasting group

This section outlines the results for the Bio-C pasting group. The hardness value after 1 month and 3 months was 47.3±0.7 KHN and 61.2±3.1 KHN in the distilled water group, and was 50.3±3.7 KHN and 62.8±1.7 KHN in the remineralization solution group, respectively.

After pasting the pulp-capping agent, hardness improved significantly after 1 month and after 3 months compared to the demineralized sample, in both

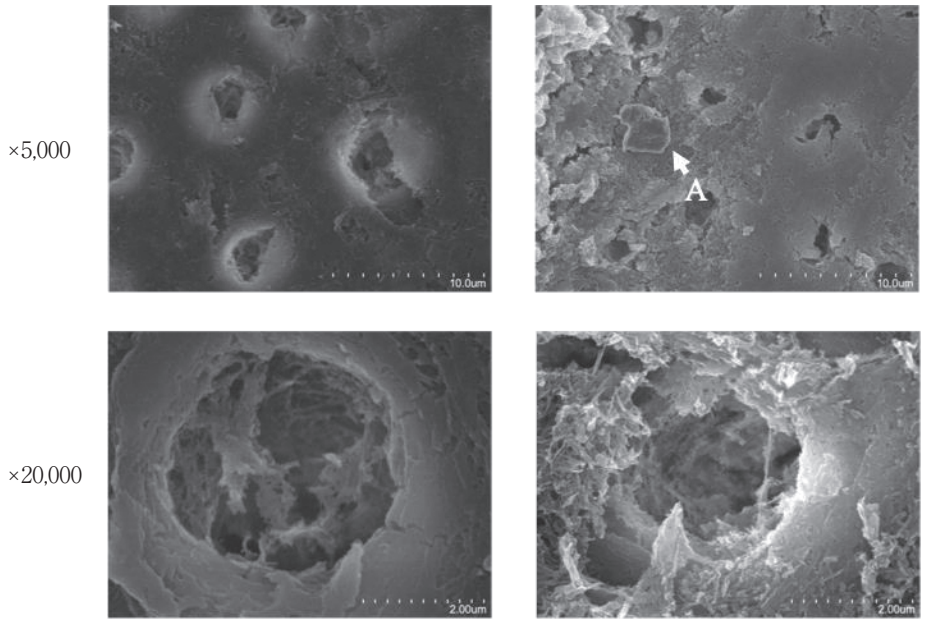

Fig. 1 SEM images of sound dentin and demineralized dentin

The surface of sound samples was covered finely with crystal deposits such as hydroxyapatite, and we were not able to observe any exposed collagen fiber.

The surface of demineralized samples showed collagen fibers exposed due to decalcification by lactic acid. Crystal deposits were not observed between collagen fibers, even though a small residual amount of crystal deposits was observed on the collagen fiber surface. A：Crystal deposits

the distilled water group and the remineralization solution group. Furthermore, it improved to a hardness level where there was no significant difference from the sound sample after 3 months in both groups.

(3) NEX-C pasting group

This section outlines the results for the NEX-C pasting group. The hardness value after 1 month and 3 months was 56.1±1.6 KHN and 64.0±0.0 KHN in the distilled water group, and was 53.1±6.1 KHN and 64.0 ±0.0 KHN in the remineralization solution group, respectively.

After pasting the pulp-capping agent, hardness improved significantly after 1 month and after 3 months compared to the demineralized sample, in both the distilled water group and the remineralization solution group. Furthermore, it improved to a hardness level where there was no significant difference from the sound sample after 1 month and after 3 months, in both groups.

(4) Control

This section outlines the results for the control group. The hardness value after 1 month and 3 months was 25.2±2.5 KHN and 22.4±0.4 KHN in the distilled water group, and was 25.1±1.7 KHN and 22.3±0.1 KHN in the remineralization solution group, respectively.

In the control group, no significant difference was observed in both groups compared to the decalcified sample in the hardness value after 1 month and the value after 3 months.

2．Observation of SEM images

1）Observation of sound samples

Figure 1 (left) shows the results of SEM image observation on sound samples. The sample surface was covered finely with crystal deposits such as hydroxyapatite, and we were not able to observe any exposed collagen fiber.

2）Observation of demineralized samples

Figure 1 (right) shows the results of SEM image observation on demineralized samples. Collagen fibers were exposed due to demineralization by lactic acid. Crystal deposits were not observed between collagen

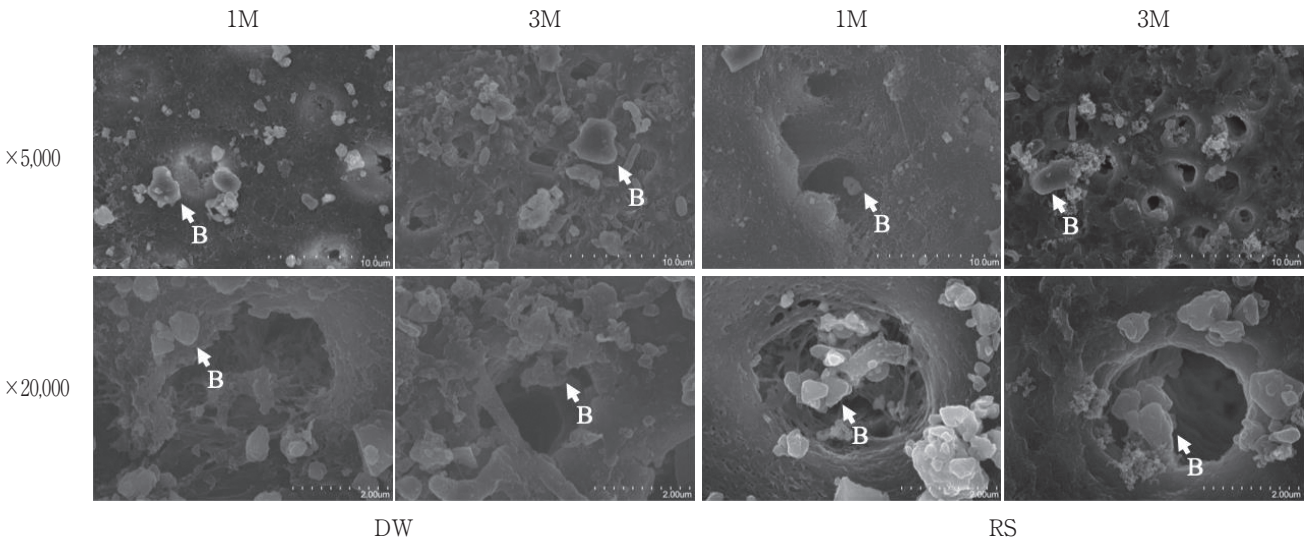

Fig. 2 SEM images of dentin applied SH-C

In both the distilled water group and the remineralization solution group, we observed that the intertubular dentin had become finer due to calcification and that fine crystal deposition had occurred at the dentinal tubule opening after 1 month and after 3 months. B：Fine crystal deposition

fibers, even though a small residual amount of crystal deposits was observed on the collagen fiber surface.

3）Observation of pulp-capping samples

(1) SH-C group

Figure 2 shows the SEM images for the SH-C group.

The observation results for the distilled water group are described below. We observed that the intertubular dentin had become finer due to calcification and that fine crystal deposition had occurred at the dentinal tubule opening after 1 month. After 3 months, we observed that the intertubular dentin had become even finer due to calcification, along with fine crystal deposition at the dentinal tubule opening.

The observation results for the remineralization solution group are described below. We observed that the intertubular dentin had become finer by calcification and that fine crystal deposition had occurred at the dentinal tubule opening in a similar fashion to the distilled water group after 1 month. After 3 months, we observed that the intertubular dentin had become even finer due to calcification, along with fine crystal deposition at the dentinal tubule opening.

(2) Bio-C group

Figure 3 shows the SEM images for the Bio-C group.

The observation results for the distilled water group are described below. We observed that the intertubular dentin had become finer due to calcification and that minute crystal deposition had occurred at the dentinal tubule opening after 1 month. After 3 months, we observed that the intertubular dentin had become even finer due to calcification, along with minute crystal deposition at the dentinal tubule opening.

The observation results for the remineralization solution group are described below. We observed that the intertubular dentin had become finer by calcification and that minute crystal deposition had occurred at the dentinal tubule opening in a similar fashion to the distilled water group after 1 month. After 3 months, we observed that the intertubular dentin had become even finer due to calcification, along with minute crystal deposition at the dentinal tubule opening.

(3) NEX-C group

Figure 4 shows the SEM images for the NEX-C group.

The observation results for the distilled water group are described below. We observed that the intertubular dentin had become finer due to calcification and that granular crystal deposition had occurred at the dentinal tubule opening after 1 month. After 3 months, we observed that the intertubular dentin had become even finer due to calcification, along with granular crystal deposition at the dentinal tubule opening.

The observation results for the remineralization solution group are described below. We observed that the

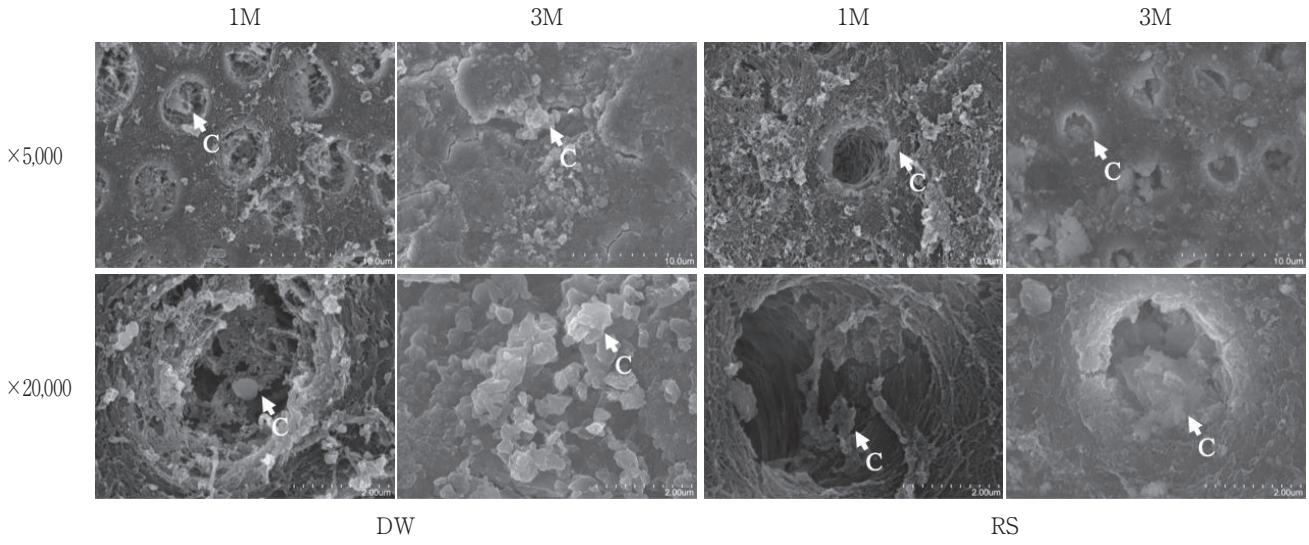

Fig. 3 SEM images of dentin applied Bio-C

In both the distilled water group and the remineralization solution group, we observed that the intertubular dentin had become finer due to calcification and that minute crystal deposition had occurred at the dentinal tubule opening after 1 month and after 3 months. C：Minute crystal deposition

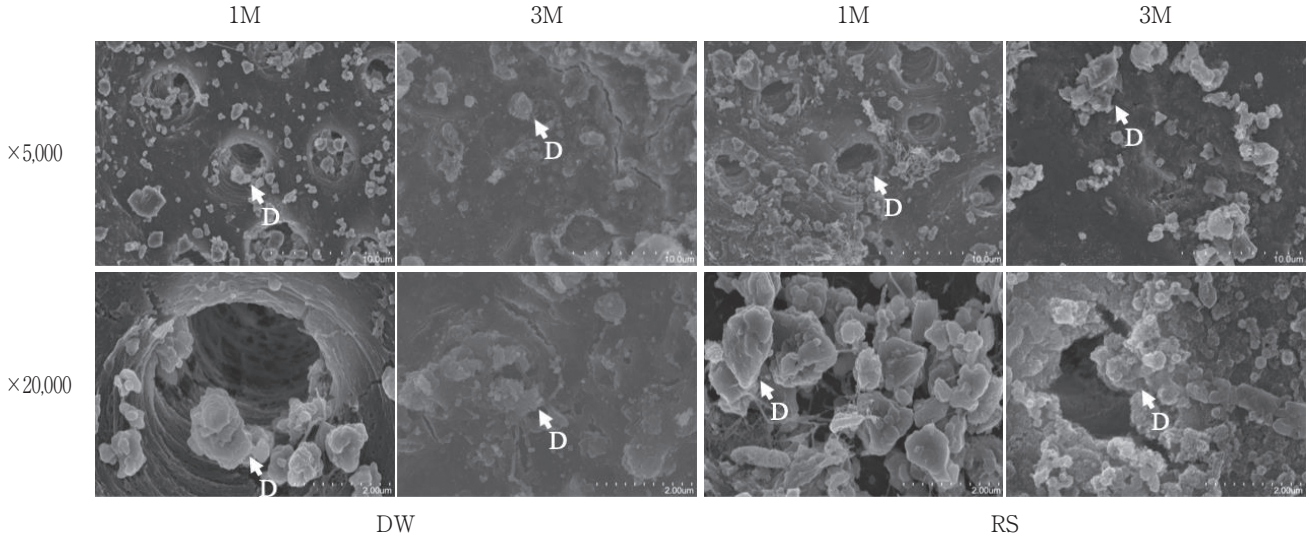

Fig. 4 SEM images of dentin applied NEX-C

In both the distilled water group and the remineralization solution group, we observed that the intertubular dentin had become finer due to calcification and that granular crystal deposition had occurred at the dentinal tubule opening after 1 month and after 3 months. D：Granular crystal deposition

intertubular dentin had become finer by calcification and that granular crystal deposition had occurred at the dentinal tubule opening in a similar fashion to the distilled water group after 1 month. After 3 months, we observed that the intertubular dentin had become even finer due to calcification, along with granular crystal deposition at the dentinal tubule opening.

(4) BC group (control)

Figure 5 shows the SEM images for the BC group.

The observation results for the distilled water group are described below. The intertubular dentin had become coarser due to decalcification after 1 month and after 3 months in a similar fashion to the demineralized sample, while exposed collagen fibers were observed

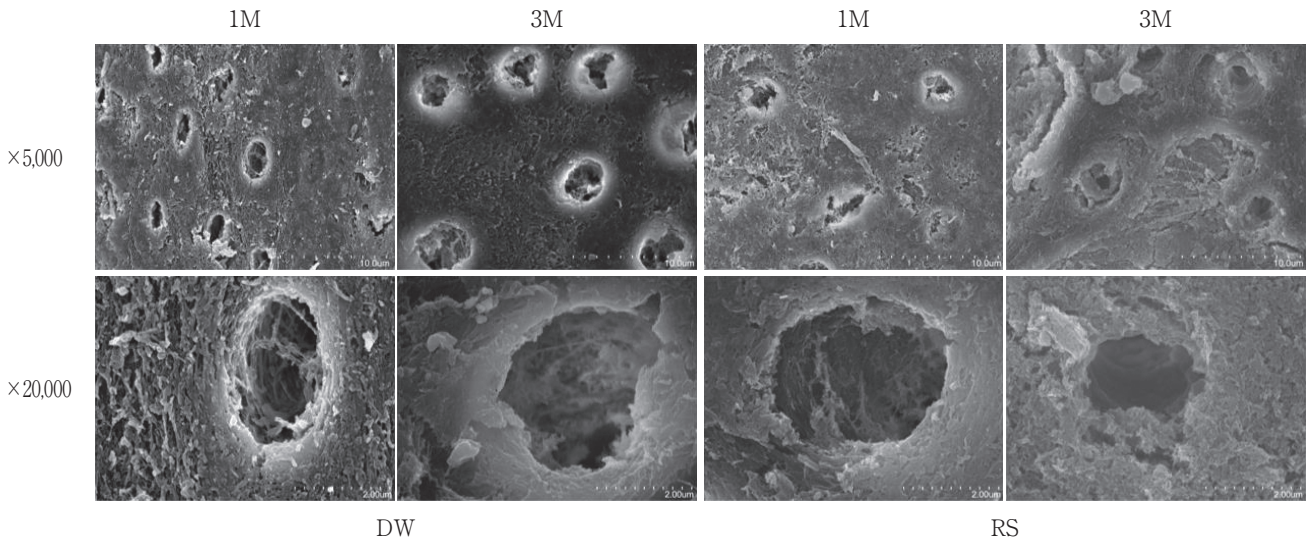

Fig. 5 SEM images of dentin applied BC

In both the distilled water group and the remineralization solution group, we observed that the intertubular dentin had become coarser due to decalcification after 1 month and after 3 months in a similar fashion to the demineralized sample, while exposed collagen fibers were observed but there was no formation of calcium deposit-like aggregates.

but there was no formation of calcium deposit-like aggregates.

The observation results for the remineralization solution group are described below. Like the distilled water group, the intertubular dentin had become coarser due to decalcification after 1 month and after 3 months in a similar fashion to the demineralized sample, while exposed collagen fibers were observed but there was no formation of calcium deposit-like aggregates.

Discussion

Cases in which the carious dentin reaches into deeper parts of dentin adjacent to the pulp are frequently encountered in clinical practice. It has been considered that pulp extirpation is recommended when pulp exposure occurs due to caries in such cases. However, recent biological examinations of pulp[21,22] have shown that pulp has a high healing ability, and that inflammation in pulp is reversible, thus highlighting the importance of pulp preservation. Therefore, atraumatic indirect pulp capping (AIPC) is recommended, in which pulp extraction is avoided but instead preservation of the pulp is attempted by intentionally leaving the infected dentin adjacent to the pulp to paste a polycarboxylate cement combined with tannin-fluoride preparation or calcium hydroxide formulation, and promoting the sterilization and remineralization of the infected dentin that was left as well as the formation of tertiary dentin (reparative dentin) even in caries that has advanced to the deep parts of dentin adjacent to the pulp. This treatment is specified as 3 months or longer in the current dental care insurance system, and so it is important to confirm the hardness after 3 months as a guide value. While the possibility of remineralization of the demineralized dentin and so forth when AIPC is conducted in dental caries treatment is important, such issues remain to be clarified. The presence of caries in removed teeth is a potential research subject for elucidating these problems. However, it is extremely difficult to secure a large number of carious teeth with dentin that has decalcified to a similar degree. Therefore, artificial preparation of demineralized dentin similar to natural caries would be invaluable for addressing such problems. While there are numerous reports on studies on the preparation of artificial carious dentin[23–31], many of these are limited to the surface layer; no report describes the preparation of an artificial carious dentin with thickness similar to that of caries.

In this study, we used lactic acid, which is an organic acid considered to be produced in large amounts by cariogenic bacteria, as the solution for decalcifying the dentin[32,33]. Furthermore, to prepare demineralized dentin with similar thickness to caries, we adopted the

method of Matsuda et al.[18] of soaking under suction from the side of the pulp cavity by using an aspirator instead of simply soaking the tooth in lactic acid solution[18]. As a consequence, we observed deterioration in Knoop hardness even on the sides of the pulp cavity. Although we examined the changes in Knoop hardness on the surface pasted with the pulp-capping agent over time in this study, we assume that it would be possible to prepare demineralized dentin with a certain thickness similar to that of carious dentin by also measuring the hardness on the sides of the pulp cavity and conducting further examinations in the future. This would greatly assist research on the depth of remineralization in carious dentin. The hardness of dentin has been studied for a long time, and has been measured with micro hardness testers such as the Mohs hardness tester, Vickers hardness meter, and Knoop hardness tester[34-38]. However, such studies were done on removed teeth; there has been no report on measurements of dentin in live teeth that are planted in the oral cavity. Cariotester, an instrument for measuring the hardness of carious dentin developed by Shimizu et al.[19,20], has been commercially available for several years. By using Cariotester and directly measuring the Knoop hardness of carious dentin inside the oral cavity, it is possible to apply an objective indicator called hardness to caries treatment. However, a potential problem with Cariotester is the safety of using a water-based ink inside the oral cavity, as the ink from a water-based ink felt pen must be applied to the tip of the arm with a depressor when taking a measurement. A further problem is complexity in use, since the sample hardness is measured by checking the depth to which the depressor is pressed in by using a microscope, as the ink at the tip of the depressor becomes lost only in the area that is pressed into the sample.

We stored the pulp-capping samples we prepared in a 100% humidity environment and in remineralization solution. It has been reported that the pulp liquid acted as a remineralization solution in a vital tooth[39]. Although the remineralization solution we used in this experiment does not perfectly match the components of saliva, we used it for our experiment in order to take into consideration the effects of Ca contained in saliva on remineralization.

The composition of the remineralization solution used was not completely consistent with that of dental pulp fluid, but we used it to investigate the influence of Ca contained in dental pulp fluid on remineralization. We tested it as a pulp-capping agent-covering material. In addition, samples covered only with the Base cement without a pulp-capping agent were prepared as a control because the influence of fluoride ions slowly released from the covering Base cement on softened dentin remineralization was considered. No increase in the hardness was noted in either the distilled water or the remineralization solution group. We considered that the effect of the storage solution was inferior to the zone of decalcification, because the layer covering the Base cement was thick.

Both Bio MTA cement and NEX MTA cement are used as pulp-capping agents, root canal filling agents and so forth in clinics. Characteristics of these MTA cements include the sustained release of calcium ions, and they have been reported to facilitate remineralization[40]. In this study, we observed an improvement in hardness of the demineralized dentin in both the distilled water group and the remineralization solution group after 1 month and after 3 months due to the sustained release of calcium ions. We also observed in SEM images that the intertubular dentin had become finer due to calcification, with minute crystal deposition. It is therefore assumed that remineralization of the demineralized dentin occurred by pasting these MTA cements.

The new bioactive glass-compounded pulp-capping agent we used in this study is a water-based paste whose main component is a bioactive glass. As a characteristic of bioactive glass, the capacity to release various ions (Sr, Al, Si, B, etc.) has been reported in addition to fluorine release and recharge in a similar fashion to glass ionomer cement[41-45]. In this study, we observed improvement in hardness of the demineralized dentin in both the distilled water group and the remineralization solution group after 1 month and after 3 months, as various ions were released in a sustained manner. We also observed in SEM images that the intertubular dentin had become finer due to calcification, with fine crystal deposition at the dentinal tubule opening. It is assumed that the demineralized dentin had recalcified as the bioactive glass-compounded pulp-capping agent was pasted. Compared to MTA cements, hardness had improved more slowly after 1 month, but had become equivalent to that of MTA cements after 3 months. It is

therefore considered that hardness improved slowly due to the sustained release of ions. It is also possible that the state of hardening in the demineralized dentin may differ between MTA cements with the capacity for sustained release of Ca ions and the bioactive glass-compounded pulp-capping agent with the capacity for sustained release of various ions; we should examine whether this improves the hardness in deeper parts of demineralized dentin. There may also be additional effects of various ions such as bacteriostatic effects on the recalcified dentin, in addition to the remineralization by calcium ions. According to a report by Matsuda et al.[18], pasting polycarboxylate cement combined with tannin-fluoride preparation, calcium hydroxide preparation and so forth did not achieve a hardness equivalent to that of sound dentin, although they observed a tendency of improved hardness. In our study, we were able to obtain hardness equivalent to that of a sound tooth in 3 months with efficient remineralization, by using a new bioactive glass-compounded pulp-capping agent or MTA cement as the pulp-capping agent. Since these agents have also been reported to have antibacterial properties, biocompatibility, and capacity to induce hard tissue formation[46–48], we expect they will be effectively used in clinical applications in the future.

Conclusions

Based on hardness measurements of various pulp-capping samples by using Cariotester, a device for measuring Knoop hardness, and observation of SEM images on sample surfaces, we obtained the following conclusions.

1. When only the back layer was covered with conventional glass ionomer cement (Base cement) instead of implementing pulp capping, there was neither an improvement in the hardness of the demineralized dentin nor a tendency for remineralization.

2. When the new bioactive glass-compounded pulp-capping agent was applied, the hardness of the softened dentin was improved and a tendency for remineralization was observed in a similar fashion to applying MTA cement.

The authors declare no conflicts of interest associated with this manuscript.

References

1) Terashima S. Differentiation of two layers of carious dentin by staining. J Stomatol Soc Jpn 1970; 37: 279–286. (in Japanese)
2) Sato Y. Removal of infected dentin using fuchsin staining as a guide: 1. Experiment with extracted carious teeth. J Stomatol Soc Jpn 1973; 40: 420–427. (in Japanese)
3) Sato Y. Removal of infected dentin using fuchsin staining as a guide: 2. Experiment with vital carious teeth. J Stomatol Soc Jpn 1974; 41: 202–211. (in Japanese)
4) Sano H. Relationship between caries detector staining and structual characteristics of carious dentin. J Stomatol Soc Jpn 1987; 54: 241–270. (in Japanese)
5) Sano H, Nakajima M, Tagami J. Diagnosis and removal of dental caries using caries detector. Yasuda N, Tagami J. Practice in prosthodontics separate volume. Search for new cariology and operative dentistry. Ishiyaku Shuppan: Tokyo; 1997. 89–92. (in Japanese)
6) Katou S. Remineralization of artificially decalcified dentin in living dog teeth. J Stomatol Soc Jpn 1968; 35: 613–625. (in Japanese)
7) Miyauchi H. Physiological remineralization of carious dentin. J Stomatol Soc Jpn 1976; 43: 384–393. (in Japanese)
8) Yamamoto Y. An in vitro study on the remineralization of inner carious dentin: The remineralization effect of glass ionomer cement. Tsurumi Univ Dent J 1984; 10: 57–74. (in Japanese)
9) The Japanese Society of Conservative Dentistry. Clinical guidelines for treating caries in adults following a minimal intervention policy—Evidence and consensus based report. 1st ed. Nagasue Shoten: Kyoto; 2009. 52–69. (in Japanese)
10) Leung RL, Loesche WJ, Charbeneu GT. Effect of dycal on bacteria in carious lesions. J Am Dent Assoc 1980; 100: 193–197.
11) Ogawa F, Machida Y. Clinical observation of pulp capping on deep carious lesions. J Tokyo Dent Soc 1984; 84: 1963–1970. (in Japanese)
12) Goto J. Clinical and histological study of indirect pulp capping on deep carious lesions. Jpn J Ped Dent 1985; 23: 926–938. (in Japanese)
13) Nagamine M. Studies on treatment of deep carious lesions utilizing polycarboxylate cement combined with tannin-fluoride preparation. J Okayama Dent Soc 1993; 12: 1–25. (in Japanese)
14) Leksell E, Ridell K, Cvek M, Mejare I. Pulp exposure after stepwise versus direct complete excavation of deep carious lesions in young posterior permanent teeth. Endod Dent Traumatol 1996; 12: 192–196.

15) Bjorndal L, Thylstrup A. A clinical and microbiological study of deep carious lesions during stepwise excavation using long treatment interval. Caries Res 1997; 31: 411–417.
16) Bjorndal L, Thylstrup A. A practice-based study on stepwise excavation of deep carious lesions in permanent teeth: a 1-year follow-up study. Community Dent Oral Epidemiol 1998; 26: 122–128.
17) Bjorndal L, Larsen T. Changes in the cultivable flora in deep carious lesions following a stepwise excavation procedure. Caries Res 2000; 34: 502–508.
18) Matsuda T, Onda K, Tanimoto H, Yoshikawa K, Yamamoto K. Study on rehardening of demineralized dentin with pulp-capping agents using a new hardness determination system. Jpn J Conserv Dent 2014; 57: 29–42.(in Japanese)
19) Shimizu A, Honda K, Natsumi Y, Hasegawa M. Development and evaluation of a practical device for detecting hardness of carious dentin. Jpn J Conserv Dent 2006; 49: 45–51.(in Japanese)
20) Shimizu A, Kinoshita N, Honda K, Abe T, Hasegawa M. A new trial for determining the hardness of carious dentin. Jpn J Conserv Dent 2008; 51: 565–570.(in Japanese)
21) Edwina K. Caries removal and the pulpo-dentinal complex. Dent Update 2000; 27: 476–482.
22) Ranly DM, Garcia-Godoy F. Current and potential pulp therapies for primary and young permanent teeth. J Dent 2000; 28: 153–161.
23) Satake S, Hasegawa M, Shimizu A, Yoshioka W. Production of a caries-like lesion and remineralization of the lesion. part 1. Caries-like lesion produced by high viscosity etching gel and rehardening of the lesion. Jpn J Conserv Dent 1990; 33: 1540–1549.(in Japanese)
24) Wefel JS, Heilman JR, Jordan TH. Comparisons of in vitro root caries models. Caries Res 1995; 29: 204–209.
25) Iijima Y, Takagi O. The reaction of enamel and root dentine samples after demineralization and remineralization. J Dent Hlth 1996; 46: 290–296.
26) Konishi M. Creation of artificial carious dentin using lactic acid and collagenase. Jpn J Conserv Dent 1999; 42: 144–157.(in Japanese)
27) Shimizu A, Maeda T, Natsumi Y, Honda K, Hasegawa M. Attempts at producing artificial caries-like lesion in dentin. part 1. An investigation on decalcification method and period. Jpn J Conserv Dent 1999; 42: 1116–1122.(in Japanese)
28) Urayama A, Yoshiyama M, Kimochi T, Matsuoka N, Ozaki K, Matsuo T. Resin infiltration into artificial carious dentin. Jpn J Conserv Dent 1999; 42: 529–535.(in Japanese)
29) Itota T, Iwai Y, Okamoto M, Tashiro Y, Nakabo S, Nishitani Y, Nagamine M, Torii Y, Yoshiyama M. Remineralization of decalcified dentin by a fluoride-releasing adhesive system. Jpn J Conserv Dent 2001; 44: 175–181.(in Japanese)
30) Shimizu A, Maeda T, Natsumi Y, Hasegawa M, Honda K. Attempts at producing artificial caries-like lesion in dentin. part 2. Decalcification using buffer solution and/or acid gel. Jpn J Conserv Dent 2001; 44: 583–589.(in Japanese)
31) Yamazaki T, Miake Y, Ishikawa T, Hiruma N, Yanagisawa N. The effect of calcified seaweed on remineralization of artificially demineralized dentin. J Hard Tissue Biol 2011; 20: 11–16.(in Japanese)
32) Hanada N. Consider etiology of dental caries as disease group: A proposal. Dental Outlook 1997; 89: 309–313.(in Japanese)
33) Nikiforuk G. Abe K, Kameyama Y, Kitano S, Sakaki T, Takeuchi H. Understanding dental caries: Etiology and mechanisms basic and aspects. 1st ed. Gakken Shoin: Tokyo; 1987. 149.(in Japanese)
34) Miake K, Higashi S, Fukuyama S, Ebihara N, Tako A, Senchi H, Nabeya S. Microhardness studies on human teeth. part 2. Microhardness of enamel and dentin of permanent molars. J Tokyo Dent Soc 1967; 67: 961–986.(in Japanese)
35) Nishimura F, Okazaki K, Kono Y, Komori K, Nomoto S. Compressive behavior and microvickers hardnes of human enamel and dentin. J J Dent Mater 1986; 5: 449–454.(in Japanese)
36) Saito H. Study on the method of hardness test and the distribution of micro-vickers hardness of human teeth. J J Dent Mater 1991; 10: 241–265.(in Japanese)
37) Willems G, Lambrechts P, Braem M, Celis JP, Vanherle G. A classification of dental composites according to their morphological and mechanical characteristics. Dent Mater 1992; 8: 310–319.
38) Akimoto N, Ohmori K, Yokoyama G, Momoi Y, Kohno A. Study on microhardness of human dentin using nano-indentation tester. Jpn J Conserv Dent 1999; 42: 96–101.(in Japanese)
39) Shellis RP. Effects of a supersaturated pulpal fluid on the formation of caries-like lesions on the roots of human teeth. Caries Res 1994, 28: 14–20.
40) Gandolfi MG, Siboni F, Prati C. Chemical-physical properties of TheraCal, a novel light-curable MTA-like material for pulp capping. Int Endod J 2012; 45: 571–579.
41) Minami K, Nagai Y, Inaba D, Someya Y, Matsuda K, Yonemitsu M. Effect of fluoride releasing restorative materials on remineralization of root dentin in vitro. J Dent Hlth 2001; 51: 293–297.(in Japanese)
42) Tominaga T, Mukai Y, Teranaka T. Time-course study

of fluoride-releasing restorative materials on dentin remineralization and acid resistance in vitro. Jpn J Conserv Dent 2007; 50: 808-817.(in Japanese)
43) Ten Cate JM, van Duinen RN. Hypermineralization of dentinal lesions adjacent to glass-ionomer cement restorations. J Dent Res 1995; 74: 1266-1271.
44) Hatibovic-Kofman S, Suljac JP, Koch G. Remineralization of natural carious lesions with a glass ionomer cement. Swed Dent J 1997; 21: 11-17.
45) Bynum AM, Donly KJ. Enamel de/remineralization on teeth adjacent to fluoride releasing materials without dentifrice exposure. ASDC J Dent Child 1999; 66: 89-92.
46) Wu D, Ma T, Fan B. Ag-loaded mesoporous bioactive glasses against Enterococcus faecalis biofilm in root canal of human teeth. Dent Mater J 2015; 34: 54-60.
47) Izumi T, Maruta M, Itaya K, Minakami M, Matsumoto N, Hatakeyama J, Nakayama H, Matsuya S, Anan H. Histopathological study on osteogenic ability of bioactive glass containing strontium. Jpn J Conserv Dent 2014; 57: 492-501.(in Japanese)
48) Oguchi H, Karube Y, Raita Y, Ohshima T, Maeda N. Responses of human gingival epithelial cells (HGE-15 cells)to RBP1 and RBP2 bioactive glass as evaluated by scanning and transmission electron microscopy. Tsurumi Univ Dent J 2014; 40: 7-17.(in Japanese)

新規バイオアクティブガラス配合覆髄剤の有効性の検討

澤井健司郎　保尾謙三　小正玲子
吉川一志　山本一世

大阪歯科大学歯科保存学講座

抄録

目的：本研究では，カリオテスターSUK-971（三栄エムイー）を用いて脱灰試料のKnoop硬さを測定し，非侵襲性歯髄覆罩（AIPC）に対する各種覆髄剤への有効性を検討した．

材料と方法：ヒト抜去大臼歯から直径10 mm，厚さ2 mmの円盤状の象牙質試料を作製し，象牙質試料の歯髄腔側からアスピレーターで吸引しながら，エナメル質側を20 mmol/*l*乳酸溶液に浸漬して，エナメル質側の硬さが20KHN程度となる脱灰象牙質試料とした．脱灰象牙質試料に，新規バイオアクティブガラス配合覆髄剤（松風），Bio MTAセメント（モリタ），NEX MTAセメント（ジーシー）を貼付し，ベースセメント（松風）で被覆したものを覆髄試料，覆髄剤を貼付せずベースセメントのみで被覆したものをコントロールとして作製し，湿度100％容器中または再石灰化溶液中で1カ月間および3カ月間保管後，軟化象牙質の硬さを測定した．試料数は各条件につき3試料とし，得られた値は一元配置分散分析およびTukeyの検定にて統計解析を行った（$\alpha=0.001$）．また硬さ測定後，覆髄剤貼付部のSEM画像の観察を行った．

結果および考察：新規バイオアクティブガラス配合覆髄剤，Bio MTAセメント，NEX MTAセメントを貼付した軟化象牙質試料では硬さが向上し，石灰化物の緻密な沈着が認められた．新規バイオアクティブガラスを配合した覆髄剤を用いることで，脱灰象牙質の硬化が認められた．

結論：バイオアクティブガラスを配合した新規覆髄剤の軟化象牙質の硬化への有効性が示唆された．

キーワード：Knoop硬さ，覆髄剤，再石灰化

責任著者連絡先：澤井健司郎
〒540-0008　大阪市中央区大手前1-5-17　大阪歯科大学歯科保存学講座
TEL：06-6910-1111，FAX：06-6943-8051，E-mail：sawai-k@cc.osaka-dent.ac.jp
受付：2019年11月15日/受理：2019年12月5日